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# Analysis of Disease Parameters in Dengue Hemorrhagic Fever versus Classic Dengue Fever Patients

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## Abstract

Dengue fever (DF) is mosquito born disease infecting 390 million individuals globally. It may present with several classical DF, dengue hemorrhagic fever (DHF) or Dengue shock syndrome. Clinical symptoms included headache, fever, bone pain or some time flue like symptoms. It is commonly diagnosed by serological detection of IgM, NS1 antigen in patients's serum. The disease progression and management is monitored by a simple complete blood picture test. This hematological test is readily available at all health care facilities. This study was designed to investigate the differentiation of hematological parameters in DF and DHF. This was a retrospective cross sectional study, performed on 158 patients. The data was collected from the Allied Laboratory Lahore, Pakistan between March 2020 to February 2022. Demographic and clinical data was analysed by SPSS V.26. Data suggest that the dengue infection was more common in active age group i.e.15-30 years of age. The data showed significance difference between of the hematological parameters in both groups (P value less than 0.05). The group comparison of hematological parameters showed TLC (DF: Mean=4.25, DHF: Mean=3.21), RBC count (DF:DHF, Mean=5.66:6.68), Platelets count (DF:DHF, Mean=129.31: 84.39) and Hb levels (DF:DHF, Mean=13.08: 14.27) and HCT (DF:DHF, Mean=51.08: 59.27). Results showed that in DHF, malfunction in intrinsic coagulation pathway is observed. DF patients might have no bleeding while DHF can have no shock or shock, resulting in DSS. The study concludes that these laboratory findings help in efficient understanding of clinical picture of dengue fever and in early identification of the disease in narrow resources areas. The development and implementation of sustainable preventive and control strategies that will reverse the trend of emerging dengue hemorrhagic fever is a serious problem for public health experts in Pakistan and the world at large.

**Keywords**: DHF, HCT, DF, Cross-sectional study, SPSS.

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## Introduction

Dengue is a mosquito (vector) borne viral disease transmitted through the female mosquito *Aedes aegypti,* although other species from this genus *aedes* can be the source of transmission of viral dengue infection as well (1). Dengue virus is a single positive-stranded RNA virus of the family *Flaviviradae* and genus *Flaviviru* (1). It has 4 well defined serotypes designate as DENV-1, DENV-2, DENV-3 and DENV-4 (2).

The mature DENV virion is characterized by smooth surface that measures around fifty nanometers, whereas the immature virion has spikes on surface and measures sixty nanometer (3). Their genome compose of 3 structural proteins which are named as C, M and E protein and 7 non-structural proteins, called as NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5 (4). Non-structural proteins are important in RNA replication, whereas structural proteins make up the components of the DENV virion (5).

In Recent Years the worldwide prevalence of dengue has grown enormously. The estimated dengue infections per year were 390 million of which ninety-six million had shown up clinically. Prior to 1970, only 7 countries had suffered dengue outbreak. But now the infection is endemic in overly hundred countries of WHO regions of the Americas, Western Pacific, the Eastern Mediterranean, Africa and the South-East Asia but America, South-East Asia and Western Pacific regions are badly hit by this disease (6). Epidemiological data of 2015 recorded 2.35 million cases in America, out of which 10200 cases were critically diagnosed with dengue resulted in deaths of 1181 sufferers. While the disease showed up word spike in 2021, with 1,182,721 reported cases (6).

WHO data reported from Pakistan showed high mortality in 2019 in Sindh (42, CFR 0.27%), Punjab (23, CFR 0.22%) and capital Islamabad (22, CFR 0.16%). Classic dengue fever is referred as an acute illness which is distinguished by the onset of high fever, body rash, headache and musculoskeletal pain. Fatal illness can be caused by DENV serotypes which may develop Dengue hemorrhagic fever (the severe form of dengue fever) and dengue shock syndrome (DSS).

The parameter that separates it from classic dengue fever is plasma leakage which results in increased vascular permeability. Frequent loss of plasma leads to the hypovolemic shock and if extensive bleeding prolonged it leads to death. These complications of the disease are linked with drastic thrombocytopenia and expanded vascular permeability (7). Raised HCT, elevated hepatic enzymes, and altered coagulation profiles are linked to reduced platelet count, which have been proven to predict the extremity of the condition.

In classic dengue fever, white blood cells count goes to less than 5000 cells/$mm^3$, platelet count less 1.5 lakh and 5-10% rise in hematocrit value. Leakage of plasma is the most crucial presentation of dengue hemorrhagic fever that is generally revealed by pleural effusion. The clinical blood findings of dengue hemorrhagic fever (DHF) distinguished by thrombocytopenia (platelet count <100,000 cells/$mm^3$), most importantly with 20% increase in hematocrit value.

Increased chances of hemorrhages are linked with the severe thrombocytopenia (8).

Biochemical and hematological disturbances are generally reported in dengue fever. Leukopenia is the most notable hematological change.

The hematological consequences showed fluctuation in blood cell counts, Hemoglobin concentration because of plasma leakage, leucopenia due to reduced number of neutrophils, thrombocytopenia, presence of atypical lymphocytes and relative lymphocytosis before shock and changes in blood hemostasis with continual presence of hemorrhagic presentation (9).

Dengue linked liver failure has increased number of death rate because of its related problems which includes extreme loss of blood, brain disease, nephropathy (10). The aim of this study was to investigate the clinical parameters of different phases of dengue infection for better patient management during classical degue fever and dengue hemorrhagic fever (DHF).

This clinical retrospective cross-sectional study was conducted on the hematological parameters of DF and DHF. The data of total 158 serologically (IgM, NS1) confirmed patients was collected from the Allied Laboratory Lahore, Pakistan between March 2020 to February 2022.The ethical approval for this study was ethical review committee of University of Chenab, Gujrat. Inclusion criteria involved serologically proven dengue patients which were previously admitted to the hospitals or had their treatments from medical centers.

All the patients who previously had disturbed hematological findings or had any hematological disease, e-g: anemia, were excluded from study. Patients with any renal, cardiac or hepatic disorder were also excluded. The demographic and clinical data was analysed by SPSS V.26. In this study all age group patients were included but most prevalent cases of degue were between the age group of 21-30 (N=53) as shown in table. 1. The study was designed to analyze the hematological differences in classic dengue fever and dengue hemorrhagic fever. To measure their variances in both groups the data of complete blood cell count was collected and statistical analysis were performed. Therefore, an independent sample t-test was performed to compare disease hematological parameter's mean scores for classic dengue fever and dengue hemorrhagic fever.

**Table 1: Age wise distribution of dengue patients**

| Age group | |
|---|---|
| **Age** | **N= 158** |
| 2-10 | 4 |
| 11-20 | 18 |
| 21-30 | **53** |
| 31-40 | 20 |

| | |
|---|---|
| 41-50 | 28 |
| 51-60 | 16 |
| 61-70 | 10 |
| 71-80 | 9 |

**Table 2: CBC parameters analysis for both groups**

| | Fever Group | N | Mean | Std. Deviation | Std. Error Mean | P- Value (T Test) |
|---|---|---|---|---|---|---|
| **TLC** | Classic Dengue Fever | 112 | 4.25 | 1.40 | 0.13 | .000 |
| | Dengue Hemorrhagic Fever | 46 | 3.21 | 1.79 | 0.26 | .001 |
| **RBC** | Classic Dengue Fever | 112 | 5.66 | 1.02 | 0.10 | .000 |
| | Dengue Hemorrhagic Fever | 46 | 6.68 | 1.28 | 0.19 | .000 |
| **Hb** | Classic Dengue Fever | 112 | 13.08 | 1.57 | 0.15 | .000 |
| | Dengue Hemorrhagic Fever | 46 | 14.27 | 1.84 | 0.27 | .000 |
| **Platelets** | Classic Dengue Fever | 112 | 129.31 | 14.93 | 1.41 | .000 |
| | Dengue Hemorrhagic Fever | 46 | 84.39 | 16.91 | 2.49 | .000 |

An independent sample t test was performed to differentiate hematological parameter TLC's mean value for classic dengue fever and dengue hemorrhagic fever and data shown in Table 2 figure 1. There was a significant variation in TLC mean for classic fever (M=4.2464) and hemorrhagic fever (M=3.2087); t= 3.899. The degree of the mean differences is quite significant (mean difference = 1.03773). An independent sample t test was performed to differentiate hematological parameter RBC's mean scores for classic dengue fever and dengue hemorrhagic fever. There was a significant variation in RBC mean for classic dengue fever (M=5.6552) and dengue hemorrhagic fever (M=6.6793); t = 5.307. The degree of the mean differences is quite significant (mean difference = 1.02417). An independent sample t test was performed to differentiate hematological parameter Hb mean scores for classic dengue fever and dengue hemorrhagic fever. There was a remarkable variation in Hb mean for classic fever (M=13.0821) and hemorrhagic fever (M=14.2761); t = 4.125. The degree of the mean differences (mean

difference = -1.19394) is very significant. An independent sample t test was performed to differentiate hematological parameter platelet mean scores for classic dengue fever and dengue hemorrhagic fever. There was a remarkable variation in platelet mean for classic fever (M=129.3125) and hemorrhagic fever (M=84.3913) ; t = 16.513. The significance of the mean differences is quite notable (mean difference = 44.92120).

**Table 3: CBC parameter analysis**

| | Fever Group | N | Mean | Std. Deviation | Std. Error Mean | P- Value (T Test) |
|---|---|---|---|---|---|---|
| **HCT** | Classic Dengue Fever | 112 | 51.08 | 6.000 | .564 | .000 |
| | Dengue Hemorrhagic Fever | 46 | 59.27 | 3.328 | .491 | |

An independent sample t test was also performed to differentiate hematological parameter hematocrit mean scores for classic dengue fever and dengue hemorrhagic fever. There was a remarkable variation in HCT mean for classic dengue fever (M=51.08) and dengue hemorrhagic fever (M=59.27); t = -10.9. The degree of mean the differences is quite notable (mean difference = 8.190). The data represented in figure-2 , Table-3.

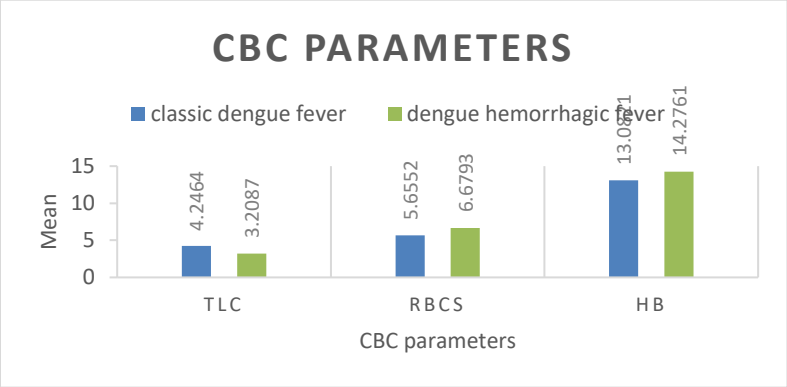
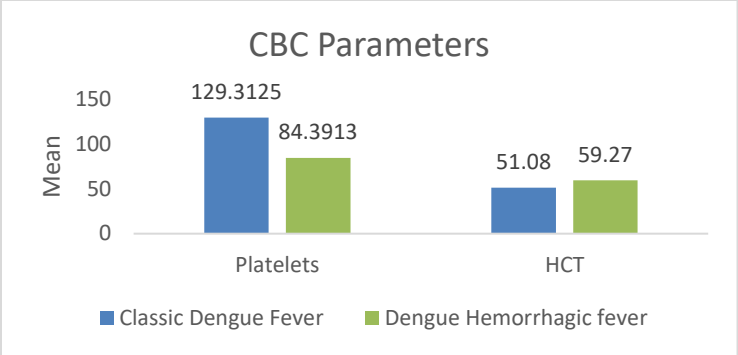



**Figure-1: Mean values of Classic Dengue fever and Dengue Hemorrhagic fever.**




**Graph 1: Mean values of Classic Dengue fever and Dengue Hemorrhagic fever.**

## Discussion

Dengue fever is a viral disease caused by the virus known as dengue virus (DENV). It is endemic in more than hundred countries. It is hard to separate the dengue from the other infectious diseases because dengue does not have any specific marker that can detect it earlier. Commnly patients present with mild symptoms like flu, headache, muscles pain, fever. If it is not treated early, it leads to its fatal form known as Dengue Hemorrhagic Fever. This research aimed to distinguish dengue hemorrhagic fever patients from the classic dengue fever patients by analyzing their hematological parameters. Hematological parameters play important role in early diagnosis of the disease in areas where resources are limited.

In one of previous studies on dengue, K Jayashree et.al claimed that decreased number of platelet count and increased vascular permeability are the two major attributes of the dengue hemorrhagic fever and are known to be as a recovery factor of dengue fever, hemorrhagic fever and dengue shock syndrome. Their study showed that seventy percent of the patients involved in their study had thrombocytopenia while the rest had normal platelet count (11).

But in our study, all the involved patients had platelet count less than 150,000 cells per millimeter cube. Majority of the cases fell between the ages of 20-50 years. Fewer cases of children were reported in this research. In this study, classic dengue fever was the most prevalent in patients than dengue hemorrhagic fever. The disease is more prevalent in males (55%) than females (45%). Among males, classic dengue fever was observed in 68% patients while 32% had dengue hemorrhagic fever. On the other hand, 75% females had classical dengue fever although 25% showed dengue hemorrhagic fever. The high prevalence in males may be due to active outdoor activities particularly during mosquitos bite timings. During dengue infection, a wide range of altered findings are seen in peripheral blood. A 20% increase in hematocrit volume is the most familiar finding of dengue hemorrhagic fever (12). Throughout the illness, a gradual reduction in granulocyte count, i-e, neutrophils lead to massive decrease in

leukocyte count. Thrombocytopenia, leukopenia are the most commonly reported findings (13).

Prevalence of dengue in our research was higher in the group people of ages between 21 to 30 years. Leukopenia and thrombocytopenia was commonly observed both classic dengue and DHF which seems consistent with studies the bone marrow suppression in case of dengue infection (11) (14). The elevated levels of hemoglobin and hematocrit were only in dengue hemorrhagic fever throughout the illness. In DHF, plasma leakage results in hemoconcentration which may develop shock (15).

In the dengue hemorrhagic variant and other severe forms, thrombocytopenia appeared from the day of getting symptoms and remained firm during the whole disease course while in classical form, thrombocytopenia appeared late. The outcomes are consistent like other literatures, which indicates DHF patients had mild or severe thrombocytopenia (16).

In one of previous studies on dengue, Zakerin Abedin claimed that less number of platelets were observed in above 40 aged people pursued with 21-30 years aged individuals which is similar to our study results. Their outcome of study exhibited platelet count (1 to 1.5 lakh per microliter) with low WBCs count (less than five thousand per microliter) were seen in eight patients as well as platelet count (1 to 1.5 lakh per microliter) with normal WBCs count in nine patients.

In five cases, platelet count less than 1 lakh with WBCs count less than 5 thousand was observed. While six patients had platelet count less than 1 lakh with normal WBCs count. Between these sixty-seven patients, classic dengue fever was seen in twenty-six percent however sixteen percent were presented with dengue hemorrhagic fever (17).

However, our study revealed, 70% patients had platelet count less than 150000 per microliter with white blood cells count normal or less than five thousand followed by 5-10% increase in hematocrit while 30% had platelet count less than 1 lakh per microliter with white blood cells lesser than five thousand along with 20% increase in hematocrit. Among these 158 cases, 112 patients had classic dengue fever whereas 46 were shown with dengue hemorrhagic fever.

Another highlight of this study was the observation of an inverse correlation between monocytes and platelet count in DHF.

It was observed that monocyte counts were high and platelet counts were low at the time shock or bleeding symptoms first appeared. In these circumstances, it was also observed that the platelet count increased while the monocyte count decreased during the healing process. Since, a similar correlation between platelet count and monocyte count throughout the acute phase and after recovery is not observed in dengue fever cases without complications, this correlation can be used as a predictor of hemorrhagic dengue. The presence of monocytosis with thrombocytopenia might notify the doctors of potential progression to severe dengue, assisting them in deciding on closer surveillance of such individuals because it is an easily accessible haematological marker. The dengue virus primarily affects monocytes, although infected monocytes

eventually undergo apoptosis to stop the virus from spreading. Thus, we suggest that increased numbers of virus-infected monocytes in DHF lead to an excess generation of cytokines and autoantibodies against platelets, which results in thrombocytopenia. The clinical state improves when the infected monocytes die off by apoptosis, which lowers the viremia and raises the platelet count.

## Conclusion

This study suggested that thrombocytopenia with onset of disease is current recognized parameter correlated with dengue hemorrhagic fever occurrence. It is discovered that the existence of bleeding symptoms is important for differentiating between DF and DHF. In DHF patients, bleeding is brought on by thrombocytopenia, which also affects bone marrow function, platelet survival, and platelet consumption. Additionally, the coagulation system may be compromised as the condition develops to DHF. In terms of this condition, DHF patients are more likely to experience various bleeding signs, which is important in separating DF from DHF. In severe dengue (shock/hemorrhage) monocytosis and thrombocytopenia are inversely correlated with clinical recovery. In order to predict outcomes, monocytosis with thrombocytopenia might be utilised as a prognostic sign. In the clinical situation of dengue fever, the presence of substantial monocytosis can be utilised as a diagnostic aid in the absence of thrombocytopenia.

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